SPERM ULTRASTRUCTURAL FEATURES OF THE BATHYAL OCTOPOD

GRANELEDONE GONZALEZI

A. ROURA*, A. GUERRA, A. F. GONZÁLEZ, S. PASCUAL

Instituto de Investigaciones Marinas. CSIC. Eduardo Cabello 6, 36208 Vigo, Spain

TEL: (+34) 986 231 930. FAX:(+34) 986 292 762

*Corresponding author: aroura@iim.csic.es

Running Title: Sperm ultrastructure of *Graneledone gonzalezi*

ABSTRACT

The fine structure of the octopod *Graneledone gonzalezi* spermatozoa is described by electron microscopy. The acrosome is the longest ever found in Octopodidae. It consists of a long striated cone surrounded by a single helix, which is defined by a numerical expression. The nucleus is rod shaped and one of the largest in Octopodidae. The nuclear fossa reaches up to the fifth part of the nucleus acting as a flagellar root due to its connection with the axoneme-coarse fibres (ACF) via the centriole. Using the morphological characteristic of the sperm, the relationship of Graneledoninae within the family Octopodidae is discussed.

Key words: systematic, spermatozoa, ultrastructure, *Graneledone gonzalezi*, cephalopod.

INTRODUCTION

The use of molecular techniques in cephalopod phylogeny has proved to be a useful tool in octopods (Carlini *et al*, 2001, Guzik *et al.* 2005). However, these attempts have led to some results that are not easily interpretable on light of other characters. On the other hand, an ideal description of a cephalopod will include morphological, meristic, ecological, ethological, and biochemical characters (Nixon 1998). Moreover, it has been recognized the importance of broad comparative analysis of taxonomic and systematic characters to construct an accurate systematic, taxonomy and phylogeny of any cephalopod taxon (Vecchione, 1998).

Spermiogenesis and sperm ultrastructure have provided important clues defining taxonomic position and phylogenetic relationships between many groups of molluscs including cephalopods (e.g. Franzén, 1955; Galangau & Tuzet 1968a, b, Longo & Anderson 1970, Hou & Maxell 1992, Healy, 1988, 1989, 1990a, b, 1993, Selmi 1996, Zhu *et al.* 2005). The present paper is focused on a member of the subfamily Graneledoninae (Octopoda: Octopodidae). The diagnostic characters of this subfamily were summarized by Voss & Pearcy (1990). Unfortunately, it was no possible to include any reference to the subfamily's sperm morphology, because no sperm data were available.

Graneledone gonzalezi Guerra, Gonzalez & Cherel, 2000 is a bathyal species, which inhabits in the upper continental shelf (510-540m) off the Kerguelen Islands (southwestern Indian Ocean). The aim of this paper is to describe the sperm morphology of this species and to compare it with that of other Octopodidae.

MATERIAL AND METHODS

Spermatophores were extracted from a preserved specimen of *G. gonzalezi* held in the collection of the Instituto de Investigaciones Marinas. The specimen (84 mm mantle length, 334 g body weight)

was bottom trawled by the “Kerguelen de Tremarec” at 510-540 m depth off Kerguelen Islands, 43º13’- 47º18S and 69º09’- 69º16’E, February 1994 (Guerra *et al.* 2000). The animal was frozen (-20ºC) on board and fixed in 4% buffered formalin in sea water for 24 hours, then preserved in 70% ethanol.

For scanning electron microscopy (SEM), a piece of spermatophore was fixed during 4 h in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.3 at 4ºC) and washed for 30 min in the same buffer. The sample was then dehydrated in a series of ethanol, critical point-dried in CO_2 using a Polaron E3000 and sputter-coated in a Polaron SC500 using 60% gold-palladium. Samples were then examined with a Philips XC30 SEM operating at 10-20 kV.

For transmission electron microscopy (TEM), sections of spermatophores were fixed in 3.0% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2 for 12 h at 4ºC, washed in the same buffer for 4 h at 4ºC and then post-fixed in buffered 2.0% osmium tetroxide for 4 h at the same temperature. After dehydration in a graded ethanol series, the fragments were embedded in Epon, sectioned with diamond knife, double-stained with uranyl acetate and lead citrate, and observed in a JEOL 100CXII TEM operated at 80 kV.

Spermatozoa measurements were taken using an imaging data processor NIS-Elements D 2.30. In order to describe the helicoidal structure of the acrosome we measured the distance between spires, which correspond to a complete turn of the helix in 16 acrosomes of the specimen. The distance between spires was compared with the number of spires using simple linear regression (Fig. 1).

RESULTS

Spermatozoa measurements are summarized in Table 1. The mature spermatozoon is approximately 971 µm long. It has a head (nucleus and acrosome) of 44 µm, and a tail or flagellum of 927 µm. The acrosome is constituted by a long striated cone, surrounded by a homogeneous material arranged in a single helix of almost 9 turns. The separation between spires significantly decreases towards the

apex (Figs. 1, 2B). In longitudinal sections, the acrosome is composed of a highly compact and electron opaque substance, which is aggregated periodically to form dense striations oriented perpendicular to the long axis of the spermatozoon (Fig. 2C). Among these striations (Fig. 2D, arrowheads), exactly at the middle, there is a less electron dense double striation that we call sub-striation (Fig. 2D, arrow). Between the striations and the acrosome membrane there is a thin space filled with homogeneous material that constitutes the spires (Figs. 2C, D). The plasma membrane is tightly connected with the acrosome membrane leaving little space to allocate the cytoplasmic substance or periacrosomal material, which appears as a thin layer of electron dense granules (Fig. 2D). Longitudinal sections of the spires show parallel striations along its length (Fig. 2A arrowheads). The junction between the acrosome and nucleus is flat, and a narrow cytoplasmic lacuna of electron lucent material can be observed (Fig. 2A, D).

The nucleus is rod shaped. The plasma membrane is electron dense and closely linked to the nuclear membrane (Figs. 2D, E). Cross sections at the nuclear fossa level, show three layers under the plasma membrane (Fig. 2E): i) An outer layer or nuclear membrane, encircling the nucleus; ii) the nucleus or chromatin layer optically very dense; and iii) the nuclear fossa, optically less dense than the previous one, surrounded by the nuclear membrane. The nuclear fossa extends from the anterior part of the centriolar fossa until, approximately, the fifth part of the nucleus (Fig. 2A).

Basally, the nucleus exhibits an invagination allowing the attachment for the tail (Figs. 2A, F). The first part of the invagination, which encircles the centriolar fossa is called neck (Fig. 2F). The former accommodates the proximal part of the flagellum and the centriole. The nuclear membrane coats the centriolar fossa and the nuclear fossa until its end (Figs. 2A, E). The centriole gives rise to the axoneme-coarse fibres complex (ACF), the axis of the flagellum (Fig. 2F).

The tail can be divided into three parts: middle, principal and end pieces. The middle piece is constituted by the ACF surrounded by the mitochondrial sheath, a fibrous sheath and the plasma membrane (Figs. 2F, G). A remarkable feature is the electron dense membrane that surrounds the

ACF (Fig. 2G, arrow). The mitochondrial sheath is composed by 9 rounded, well-defined and elongated mitochondria that run parallel to the ACF axis (Figs. 2F, G). Plasma membrane and fibrous sleeve are folded on in the distal region of the middle piece forming a cylindrical, optically dense and smooth structure, the annulus (Figs. 2F, H). At its apex, it has a constriction that limits the mitochondrial sheath (Fig. 2F, arrow). No mitochondrial sheath is present at the principal piece (Figs. 2A, F). The principal piece's diameter gradually diminishes towards the end piece, due to the reduction of the coarse fibres.

DISCUSSION

In the present paper, the acrosome helix was defined with a numeric expression (Fig. 1). From our point of view, this kind of description provides an accurate tool to define the complexity of the acrosome.

Comparison of the acrosome lengths of the different Octopodidae studied to date (Galangau & Tuzet 1968, Longo & Anderson 1970, Martin *et al.* 1970, Maxwell 1974, Selmi 1996, Zhu *et al.* 2005, Roura *et al.* in press) reveals that the acrosome of *G. gonzalezi* is the longest ever reported. Morphologically, it resembles those of Octopodinae (Galangau & Tuzet 1968a, Longo & Anderson 1970, Martin *et al.* 1970, Zhu *et al.* 2005), because it is constituted by a single helix surrounding the acrosome. This feature distinguishes Graneledoninae from Bathypolypodinae, since the former has a double helix surrounding the acrosome (Roura *et al.* in press). It can also be used to differentiate between Graneledoninae and Eledoninae, whose acrosome is totally torsioned (Maxwell 1974, Selmi 1996). The extension of the inner cone until the top of the acrosome is a feature shared by Octopodinae, Bathypolypodinae and Graneledoninae. In contrast, the above extension is shorter in Eledoninae. Furthermore, the inner cone can be used as a character to distinguish between cirrates and incirrates. The incirrate octopods have an inner cone with striations oriented perpendicularly to

the long axis of the spermatozoon (Galangau & Tuzet 1968a, Leik 1970, Longo & Anderson 1970, Healy 1989, Selmi 1996, Ribes *et al.* 2002, Zhu *et al.* 2005, Roura *et al.* in press). However, cirrate octopods do not have this inner cone, though they show few (two or three in *Opisthoteuthis persephone*) striations at the base of the acrosome (Healy, 1993).

The nuclear length is a helpful character to discern between the different subfamilies of Octopodidae. The subfamily with the largest nucleus is Bathypolypodinae, where the nucleus reaches from 66 up to 86 μm (Roura *et al*. in press) followed by Eledoninae, with nuclear lengths ranging from 37.5 up to 40 μm (Selmi, 1996). Among the families studied, Graneledoninae, with its 33.9 μm, and Octopodinae with lengths from 10 up to 21 μm, are those with smallest nucleus (Galangau, 1968 b; Zhu et al. 2005).

Another feature of interest is the nuclear fossa. Its length and connection with the centriole can also be used to distinguish subfamilies. The connection between the nuclear fossa and centriole is mediated by a thin lumen in Octopodinae (Healy 1989, Longo & Anderson 1970, Zhu *et al.* 2005) and Bathypolypodinae (Roura *et al*. in press). Furthermore, it is possible to distinguish between both subfamilies, because in Bathypolypodinae the nuclear fossa is extended almost until the nuclear apex, while in Octopodinae it only reaches the forth part of the nucleus. In contrast, *G. gonzalezi* lacks this thin lumen and the nuclear fossa only reaches the fifth part of the nucleus. On the other hand, Graneledoninae can be distinguished from Eledoninae, because in the former the nuclear fossa is extremely short and has microtubules (Maxwell 1974, Selmi 1996), a feature shared with the Opisthoteuthidae (Healy 1993).

On the whole, although more studies are needed, we support the evidence noted Healy (1990a, 1993, 1995) that spermatozoa are a valuable character in cephalopod systematic studies, in a similar way than those considered to date, such as radula, statoliths and beaks (Nixon 1998).

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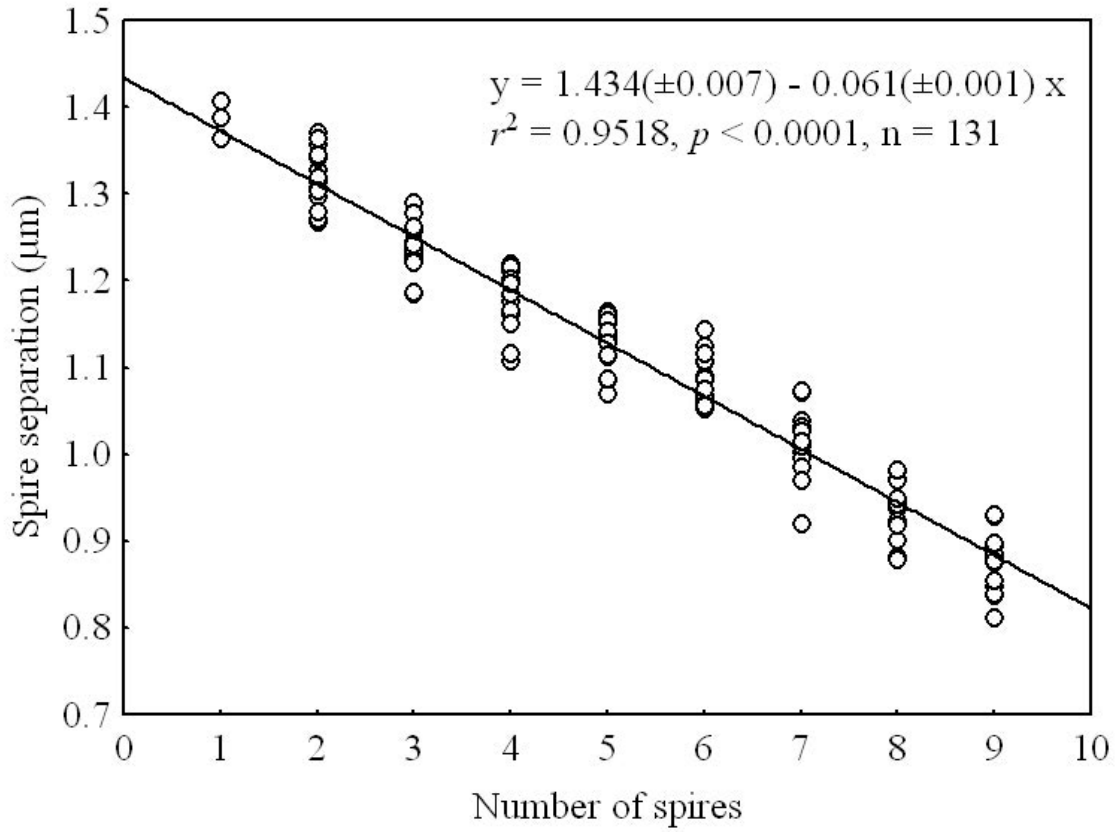
Table I. — Sperm cell measurements (µm, unless stated) of *Graneledone gonzalezi*. Mean, standard deviation (SD) and number of spermatozoa measured (n).

Measurements	*Mean, SD & n*
Sperm cell length [1]	971.4 ± 52.6 (n=8)
Head length [2]	43.81
Tail length	927.59
Acrosome (Acr.) length	9.89 ± 0.46 (n=15)
Acr. max width	0.53 ± 0.02 (n=16)
Acr. min width	0.17 ± 0.01 (n=16)
Acr. striation separation	56.8 ± 1.7 nm (n=23)
Acr. sub-striation separation	28.4 ± 1.5 nm (n=22)
Spire striation	26.5 ± 3.6 nm (n=39)
Angle of spires	47.0 º ± 2.62º (n=22)
Nucleus length	33.92 ± 1.58 (n=10)
Nucleus top width	0.53 ± 0.03 (n=11)
Nucleus posterior width [3]	0.85 ± 0.03 (n=10)
Nuclear membrane	20.0 ± 2.1 nm (n=20)
Nuclear fossa length	6.29 ± 0.34 (n=9)
Neck length	1.58 ± 0.09 (n=5)
Neck width	0.61 ± 0.05 (n=10)
Middle piece length	7.38 ± 0.43 (n=17)
Middle piece width	0.67 ± 0.04 (n=15)
Annulus length	1.26 ± 0.04 (n=5)
Annulus total width	0.64 ± 0.02 (n=14)
Annulus constriction width	0.38 ± 0.07 (n=4)
Axoneme-coarse fibres complex	0.33 ± 0.01 (n=13)
Principal piece diameter	0.41 ± 0.01 (n=11)

[1] Measures obtained from broken sperm cells
[2] Acrosome length + Nucleus length
[3] Measured at the level of the condensed chromatin

Fig. 1. – Plot of the distance between spires (μm) against number of spires measured from 16 acrosomes



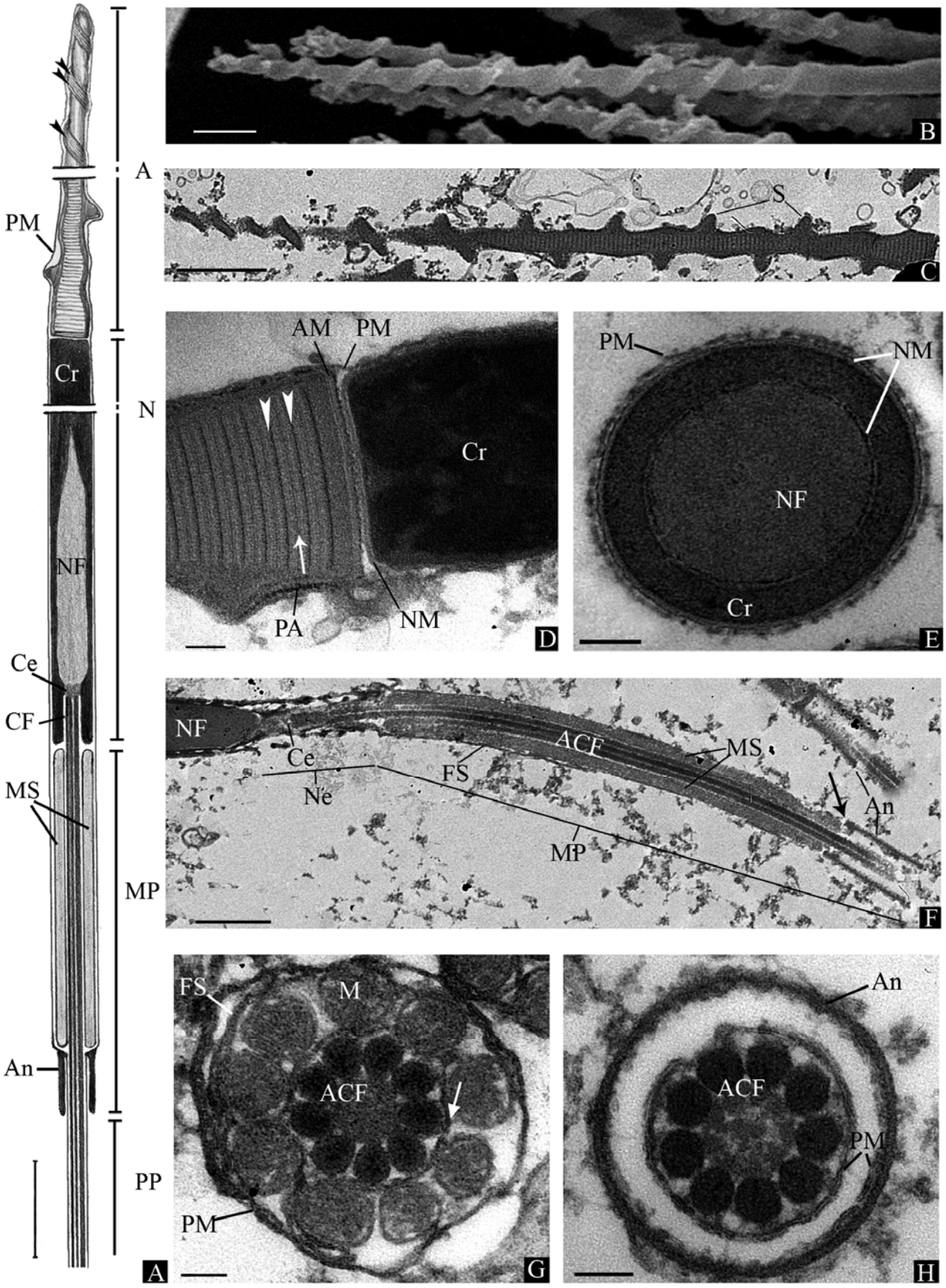

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Fig. 2**.** – A. *Graneledone gonzalezi* picture showing the sperm organization. Arrowheads: parallel striations along the spires. B. Scanning electron micrograph of the acrosome. C**.** Transmission electron micrograph (TEM) of the acrosome in longitudinal section showing the periodic striations. D**.** Longitudinal section of the acrosome-nucleus junction. Arrowheads: periodic striations, arrow: double sub-striations (TEM). E. Nucleus cross section at the nuclear fossa level (TEM). F. Longitudinal section through the neck and middle piece. Arrow: annulus constriction (TEM). G. Middle piece cross section. Note the membrane (arrow) that surrounds the axoneme-coarse fibres complex (TEM). H. Annulus cross section (TEM). Abbreviations: A, acrosome; AM, acrosome membrane; ACF, axoneme-coarse fibres complex; An, annulus; Ce, centriole; CF, centriolar fossa; Cr, chromatin; FS, fibrous sheath; MP, middle piece; M, mitochondria; MS, mitochondrial sheath; Ne, neck; NF, nuclear fossa; NM, nuclear membrane; N, nucleus; PA, periacrosomal material; PM, plasma membrane; PP, principal piece; S, spires. Scale bars: A = 2 µm; B = 1 µm; C = 2 µm; D = 0.2 µm; E = 100 nm; F = 1 µm; G = 100 nm; H = 100 nm.